COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Stuart A. Aaronson of Mount Sinai Medical Center, New York, New York, United States of America, declare as follows:

1. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to serve as a scientific consultant in connection with the Ludwig Institute for Cancer Research Opposition to the issuance of HGS Australian Patent Application 696764, in the name of HGS, entitled: "Vascular Endothelial Growth Factor-2" ("the HGS patent specification").

2. In acting as a scientific consultant for HGS, I provided a Statutory Declaration executed December 14, 2000 ("Aaronson Declaration I") in connection with the Opposition of the HGS patent specification, in which I provided my comments and opinions on what the HGS patent specification would provide to one skilled in the field of molecular biology of growth factors, *e.g.*, a post doctorate or Ph.D. candidate in a research laboratory, and to provide my comments and opinions on the experimental evidence provided in Dr. Alitalo's Statutory Declaration executed on February 15, 2000 ("Alitalo Declaration I"). I have now been asked by the Patent Attorneys representing HGS to review and provide comments on the Statutory Declaration executed by Dr. Alitalo on September 14, 2001 ("Alitalo Declaration II").

3. The HGS patent specification relates to the identification and characterization of a new member of the PDGF/VEGF family of growth factors, VEGF-2, which is also known as VEGF-C. VEGF-2 and VEGF-C are recognized to be identical proteins by those working in the field, as they have nearly identical sequences as demonstrated by the sequence alignment, appendixed hereto as Appendix I.

4. In providing my comments and opinions, I have been asked to keep in mind what the HGS patent specification would provide to one familiar with the molecular biology of growth factors as of the earliest filing date of the HGS patent specification, which I have been told is March 1994. For purposes of this analysis, I considered and give an opinion on not only what I knew and appreciated at the relevant time, but also what was expected to be known by one skilled in the field of the molecular biology of growth factors, such as graduate students and postdoctoral fellows who were in my laboratory at the relevant time. The opinions I express are, unless I state to the contrary, opinions based upon these considerations and they would have been applicable as of March 1994, the filing date of the HGS patent specification, as of September 1995, the publication date of the HGS patent specification and are also applicable now. Further, despite the fact that many of my opinions have been presented in the present tense for the ease of expression, I would have held those opinions in March 1994.

5. Dr. Alitalo attempts to provide new experimental data that allegedly addresses the criticisms raised by the HGS scientific consultants of the experimental evidence provided in Alitalo Declaration I. Dr. Alitalo further purports to analyze the expression, proteolytic processing, and secretion profiles of VEGF-2 as taught in the HGS patent specification. However, due to numerous defects in the design, performance, and analysis of these new experiments, the results presented in Alitalo Declaration II fail to provide any

meaningful information regarding the expression, processing and secretion of VEGF-2 as taught in the HGS patent specification. For example, I have identified the following deficiencies in Alitalo Declaration II:

- The failure to follow the teachings of the HGS patent specification as a whole when designing the new experiments;
- The inability to refute that, by following the teachings of the HGS patent specification, the 350 amino acid VEGF-2 protein results in the correct expression, secretion, and processing of the mature form of VEGF-2;
- The failure to design experiments to result in meaningful conclusions with respect to the teachings of the HGS patent specification; and
- The failure to draw credible or consistent conclusions based on the new experiments regarding the expression, processing and secretion of VEGF-2 as taught in the HGS patent specification.

The following paragraphs detail these deficiencies identified in the Alitalo Declaration II.

The Alitalo Declaration II Fails to Follow the Teachings of the HGS Patent Specification as a Whole

6. Dr. Alitalo purports that his new experiments were designed to determine whether utilizing the teachings provided in the HGS patent specification, the 350 amino acid form of VEGF-2 is expressed, processed and secreted. However, the experimental procedures set out in Alitalo Declaration II fail to correct one of the critical flaws of Alitalo Declaration I, *i.e.*, it fails to follow the teachings of the HGS patent specification. In particular, Dr. Alitalo's new experimental design is flawed because he fails to recognize that the HGS patent specification specifically provides that the 350 amino acid sequence may be expressed with a heterologous signal sequence.

7. Likewise, Dr. Alitalo fails to recognize that the experiments described in the Statutory Declaration executed by Susan Powers on December 20, 2000 ("Power Declaration I") demonstrated that VEGF-2 could be expressed, processed and secreted. In particular, Dr. Power's experiments demonstrated that the 350 amino acid form of VEGF-2 can be expressed, processed and secreted from cells when attached to a heterologous signal sequence as specifically taught by the HGS patent specification. (*see* HGS patent specification at page 14, lines 6 to 19).

8. In particular, Dr. Alitalo fails to acknowledge that the HGS patent specification specifically teaches the construction of expression vectors which comprise the 350 amino acid sequence of VEGF-2 fused in frame with a signal sequence. The HGS patent specification provides:

> Generally, recombinant expression vectors will include . . . . [t]he heterologous structural sequence [is] assembled in appropriate phase with translation initiation and termination sequences, and preferably, *a leader sequence* capable of directing secretion of translated protein into the periplasmic space or extracellular medium. (*see* the HGS patent specification at page 14, lines 6 to 19, emphasis added).

9. Dr. Alitalo has ignored the teaching of the HGS patent specification and has taken the position that no one familiar with the molecular biology of growth factors would utilize a heterologous signal sequence to achieve secretion of a secretory protein which has been identified to have a putative signal sequence. By following the teachings of the HGS patent specification, I believe I or a skilled molecular biologist could utilize a heterologous signal sequence in order to express, process and secrete the 350 amino acid form of VEGF-2.

10. Indeed, contrary to Dr. Alitalo's statements in Alitalo Declaration II, utilizing a heterologous signal sequence is clearly the approach that would be taken by

one familiar with the molecular biology of growth factors. One familiar with the molecular biology of growth factors equipped with the HGS patent specification would recognize that the 350 amino acid polypeptide is a secreted growth factor, and that if the 350 amino acid sequence did indeed contain a signal sequence such a sequence does not have the typical conserved motif of a signal sequence. Thus, if upon expression of the 350 amino acid form of VEGF-2, secretion of the protein did not occur, a skilled molecular biologist would utilize a strong signal sequence to ensure expression and secretion of the protein. Thus, Dr. Alitalo has taken a position which not only is clearly inconsistent with the teachings of the HGS patent specification, but is also contrary to the approach that the skilled molecular biologist would utilize.

11. Dr. Alitalo not only clearly ignores the literal teaching of the HGS patent specification which describes VEGF-2 as a secreted growth factor (*see, e.g.* HGS patent specification at page 5, lines 25 to 33), but incorrectly attributes this recognition to his own research conducted two years subsequent to the filing date of the HGS patent specification (*see* Alitalo Declaration II ¶ 2.2). Contrary to Dr. Alitalo's statements in Alitalo Declaration II, the HGS patent specification filed March 1994 accurately characterizes VEGF-2 as another member of the PDGF/VEGF family of secreted growth factors.

12. In summary, Dr. Alitalo's failure to follow the teachings of the HGS patent specification in designing the experiments provided in Alitalo Declaration II prevents any meaningful conclusions from being drawn about the expression, proteolytic processing and secretion of VEGF-2 as taught in the HGS patent specification.

The Alitalo Declaration II Fails to Refute that by Following the Teachings of the HGS Patent Specification the 350 Amino Acid VEGF-2 Protein Results in the Correct Expression, Secretion, and Processing of the Mature Form of VEGF-2.

13. In my opinion, Dr. Alitalo fails to provide experimental evidence to contradict the fact that the 350 amino acid VEGF-2 fused in frame with a heterologous signal sequence results in the expression, secretion and proteolytic processing of a mature form of VEGF-2.

14. In Alitalo Declaration II, Dr. Alitalo *newly* raises the issue that the HGS patent specification does not provide a description of the molecular weight of the approximately 30 kDa doublet. (*see* Alitalo Declaration II, ¶5.6).

15. One knowledgeable in the field of molecular biology would not require that the HGS patent specification provide the molecular weight of the secreted form of VEGF-2. Clearly, in following the teachings of the HGS patent specification, as demonstrated by Power Declaration I and Susan Power's Second Declaration ("Power Declaration II"), I or a molecular biologist would recognize that the 350 amino acid sequence of VEGF-2 can be processed to a mature form of VEGF-2 by the cell. Therefore, a molecular biologist provided with the teaching of the HGS patent specification would be able to express VEGF-2 as its naturally processed mature form. Further, a molecular biologist provided with the teaching of the HGS patent specification would recognize that the molecular weight of the resulting processed mature form of VEGF-2 is an intrinsic and natural property of that molecule.

16. Besides molecular weight, the amino acid sequence of a particular protein is also an inherent property of that protein. Statements that Dr Heldin, an apparent colleague of Dr. Alitalo, has made in support of the prosecution of Dr. Alitalo's U.S. Patent No. 6,221,839 are in agreement with this conclusion.

In particular, Dr. Heldin has stated: "It is fundamental biochemistry that polypeptides are organic chemical compounds, albeit sometimes large and complex ones. Like all organic chemical compounds, polypeptides may be characterized by any of *several inherent physical properties, such as molecular formula and molecular weight*. Such physical properties are inherent characteristics of organic molecules in that they are intrinsic properties of the molecules. Because polypeptides are themselves composed of covalently-bonded chains of smaller organic moieties called amino acids (of which there are about 20 naturally occurring), it is conventional to express the molecular formula of polypeptides as an amino acid sequence. *The amino acid sequence of any polypeptide is an inherent property of that polypeptide*." (*see*, the Declaration by Dr. Carl-Henrik Heldin, executed June 4, 1997, provided to the U.S. Patent & Trademark Office during prosecution of U.S. Patent No. 6,221,839, the "Heldin Declaration", at page 6, emphasis added).

17. Thus, I or a molecular biologist following the teachings of the HGS patent specification to express the 350 amino acid VEGF-2 polypeptide would recognize that the molecular weight and the amino acid sequence of the naturally processed and secreted form of VEGF-2 is an inherent feature of that polypeptide. Hence, it would be unnecessary for the HGS patent specification to have reported the molecular weight of the 30kDa doublet and 23kDa secreted forms of VEGF-2, because I or a molecular biologist, just like Dr. Heldin, would recognize that the molecular weight and the amino acid sequence is an inherent property of the 350 amino acid form of VEGF-2.

18. Additionally, the Alitalo Declaration II *newly* raises the issue that the processing of the 350 amino acid protein as taught by the HGS patent specification would be incorrect. (*see e.g.*, Alitalo Declaration II ¶ 3.7 or ¶ 5.5).

19. The only information and signals required by a host cell to express and process VEGF-2 to its mature form is contained in the amino acid sequence of VEGF-2. Any given host cell, *i.e.,* a mammalian host cell, will have the proteolytic enzymes and cellular machinery to naturally process VEGF-2 to its mature form. Thus, following the teachings of the HGS specification, the 350 amino acid VEGF-2 polypeptide is naturally and intrinsically processed to its mature form, as demonstrated by the results presented in Power Declaration I and II.

20. I have reviewed and agree with the characterization of the inherent features of the processing of a biologically active mature form of VEGF-2 as provided by Dr. Alitalo himself in portions of the file histories of U.S. Patent Nos. 6,221,839 and 6,245,530.

21. Accordingly, Dr. Alitalo has characterized the processing of VEGF-2 from various cell types, including both mammalian and insect expression systems and has observed that VEGF-2 is intrinsically and naturally processed to its mature form in a wide variety of cell types. (*See,* Declaration by Dr. Kari Alitalo, provided to the US. Patent & Trademark Office on June 10, 2000, in connection with prosecution of U.S. Patent No. 6,245,530, issued June 12, 2001). Evidence of the intrinsic and natural processing of the polypeptide is further confirmed by the observations that the expression of the full length and portions of VEGF-2 in various cell types results in the processing and secretion of a biologically active mature form of VEGF-2. Indeed, Dr. Alitalo has observed that the expression of polypeptides corresponding to residues 104 to 213 and 112 to 419 of the full length VEGF-2 polypeptide are correctly processed to mature forms of VEGF-2 that retain VEGF-2 biological activity. (*See*, Declaration by Dr. Kari Alitalo, provided to the U.S. Patent & Trademark Office on December 1, 1997, in connection with the prosecution of U.S. Patent No. 6,245,530; *see also*, Declaration by Dr. Kari Alitalo, provided to the U.S. Patent & Trademark Office on July 24, 2000, in connection with

the prosecution of U.S. Patent No. 6,221,839; the file histories of both U.S. Patents are annexed hereto as Appendices II and III, respectively).

22. Thus, as Dr. Alitalo has concluded, I or a molecular biologist would also conclude that the full length and portions of the VEGF-2 polypeptide are intrinsically and naturally processed to a biologically active mature form. Likewise, when appropriately expressed as taught by the HGS patent specification, the 350 amino acid form of VEGF-2 contains all of the signals required for the processing of the protein to its biologically active mature form.

The Alitalo Declaration II Fails to Design Experiments that Allows for Meaningful Conclusions with Respect to the Analysis of VEGF-2 as Taught in the HGS Patent Specification.

23. Dr. Alitalo indicates that the experiments reported in Alitalo Declaration II confirm the results of the experiments in Alitalo Declaration I and eliminate any criticisms of the experimental design described in Alitalo Declaration I. However, the criticisms of the experimental design described in Alitalo Declaration II have not been addressed because the experimental design reported in Alitalo Declaration II is also flawed and cannot support any conclusions made with respect to the expression, secretion and processing of VEGF-2 as taught in the HGS patent specification. In particular, the experiments reported in Alitalo Declaration II fail to include experimental controls to address any potential problems with expression vectors, cells, transfection, and conditions and parameters which might affect the comparative analysis of the 350 amino acid VEGF-2 and the 419 amino acid VEGF-2. I discuss these faulty experiments below.

24. Dr. Altilo's conclusions regarding the level of expression of 350 amino acid form of VEGF-2 as compared to the expression of 419 amino acid form of VEGF-2 are meaningless because no experiments were conducted to

determine the transfection efficiency of the plasmids used -- thus preventing the drawing of any valid quantitative comparisons regarding expression efficiencies from the data obtained. Disparate parameters such as cell densities or growth conditions can affect the transfection efficiency of expression constructs into cells. The transfection efficiency will directly correlate with the level of protein expression detected. Clearly if fewer cells contain the construct, fewer cells will express the protein encoded by the construct. Accordingly, any differences in the relative transfection efficiency of the VEGF-2 plasmids utilized in Dr. Alitalo's experiments would affect the comparative detection of levels of VEGF-2 protein. Dr. Alitalo does not provide experiments to demonstrate that following transfection, the same percentage of cells contained each expression construct. In fact, if the transfection efficiency of the 350 amino acid form of VEGF-2 were very low, very few cells would produce protein expressed from the construct and expression and secretion of VEGF-2 protein would be difficult to detect. Without determining transfection efficiency, any conclusion about levels of protein expressed and detected is meaningless.

25. Dr. Alitalo also speculates that the VEGF-2 as taught in the HGS patent specification is not secreted, but rather is rapidly degraded in cells. (*see* Alitalo Declaration II ¶ 3.9). This speculation has not been affirmatively confirmed by any of the experimental results described in Alitalo Declaration II. Assuming *arguendo* that normally secreted VEGF-2 is rapidly degraded in the cell, then any additional time period that passes before cells are assayed for the presence of VEGF-2 protein would result in the inability to detect the presence of VEGF-2 protein. Furthermore, the experimental protocol described in Alitalo Declaration II does not allow for detection of VEGF-2 protein expression over various time points. Rather, protein levels are assessed fifty hours post-transfection (forty-eight hours and overnight metabolic labeling). Without allowing for the detection of VEGF-2 protein that is purportedly expressed yet degraded with the passage of time over

various periods of time, the conclusions of Dr. Alitalo regarding expression and degradation are merely speculative.

The Alitalo Declaration II Fails to Draw Credible Conclusions with Respect to the Expression, Secretion and Processing of VEGF-2 as Taught in the HGS Patent Specification.

26. In summary, the flaws introduced into the experimental design and protocols of Dr. Alitalo render the experimental results inconclusive. As discussed above, in the absence of appropriate controls, comparative analysis is meaningless because disparate conditions and parameters will affect the expression, secretion and processing profiles of 350 amino acid VEGF-2 and 419 amino acid VEGF-2. In any comparative analysis, results are meaningless without the assurance that unnecessary variables are eliminated. The failure to include basic experimental controls to ascertain that there would be no problems with the expression vectors, cells, transfection efficiency, growth conditions or other parameters which affect any comparative analysis of 350 amino acid VEGF-2 and 419 amino acid VEGF-2 precludes making any meaningful conclusions.

The Alitalo Declaration II Fails to Recognize that the 350 Amino Acid VEGF-2 Is Provided by the HGS Patent Specification

27. Alitalo Declaration II purports to provide a sequence analysis of the VEGF-2 clone deposited with the American Type Culture Collection as ATCC Accession Number 75698. Based on his analysis, Dr. Alitalo alleges that the deposited clone does not have the complete 350 amino acid sequence when compared to the sequence set forth in Figure 1 of the HGS patent specification. However, in my opinion given that Figure 1 of the HGS patent specification does contain the complete 350 amino acid coding sequence, as

even Dr. Alitalo agrees, I fail to see the criticality of Dr. Alitalo's sequence data.

28. Power Declaration II clearly demonstrates that even assuming *arguendo* that the DNA deposited in ATCC Deposit No. 75698 was missing the first 24 amino acids ("the 326 amino acid form of VEGF-2"), both the 350 amino acid form and the 326 amino acid form are processed to the same molecular weight species as compared to the 419 amino acid form of VEGF-2. When expressed as taught by the HGS patent specification, the 350 amino acid form and the 326 amino acid form are both processed to a protein which resolves as a doublet at approximately 30 kDa, as does the 419 amino acid form. (Power Declaration II at ¶ 31). I note that the experimental design of Power Declaration II includes appropriate controls to address transfection efficiencies, sequence confirmation of expression constructs, and controls eliminating any disparities in cell densities or growth conditions, as would be the standard practice of a skilled molecular biologist.

29. Furthermore, the results presented in Power Declaration II clearly evince that even if the DNA deposited in ATCC Deposit No. 75698 did not contain the complete nucleotide sequence provided in Figure 1 of the HGS patent specification, I or a skilled molecular biologist, would have been able to generate the complete 350 amino acid coding sequence based on the nucleotide sequence provided in Figure 1 of the HGS patent specification in combination with standard recombinant techniques known as of March 1994. An example of an approach I could possibly use is the same approach actually used in Power Declaration II, that is, using the sequence provided in Figure 1 of the HGS patent specification, a double stranded oligonucleotide containing the missing sequence is synthesized and is ligated to the DNA obtained from the ATCC, thus recreating the coding sequence of the 350 amino acid form. Alternatively, the template for the PCR reaction could have been obtained by reverse transcription, a technique readily available in March, 1994, using RNA

from sources provided by the HGS patent specification, such as, early stage human embryo osteoclastomas, adult heart or several breast cancer cell lines (*see*, the HGS patent specification at page 5, lines 19-24, Example 1 and Figure 5). Additionally, I or a skilled molecular biologist, could follow the teachings of the examples provided in the HGS patent specification and obtain the nucleotide sequence encoding either the 419 or the 350 amino acid form of VEGF-2 from a cDNA library derived from early stage human embryo week 9 (*see*, the HGS patent specification at page 5, lines 19-24).

CONCLUSIONS

30. In sum, the experiments reported in Alitalo Declaration II are not designed to accurately assess the expression of 350 amino acid VEGF-2 as taught in the HGS patent specification. I additionally note that Dr. Alitalo's mischaracterization of the expression profile of VEGF-2 as taught in the application, based on the data generated from experimental design without basis in the teachings of the application is exaggerated by the additional flaws introduced into his experimental protocol. Finally, I note that I am in agreement with Dr. Alitalo's and Dr. Heldin's comments presented in the course of obtaining his own VEGF-2 patents which reflect Dr. Alitalo's recognition that the 350 amino acid VEGF-2 can be naturally processed to its mature form and that the molecular weight and the amino acid sequence of the resulting processed mature form of VEGF-2 are intrinsic and natural properties of that molecule.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Dr. Stuart Aaronson, _____ at New York, New York, on this 22nd day of March 2002;

before me _____

Notary Public

GEAN ROTMISTRENKO
Notary Public, State of New York
No. 41-4778718
Qualified in Queens County
Certificate Filed in New York County
Commission Expires October 31, 2005

SEQUENCE ANALYSIS DEMONSTRATING VEGF-C AND VEGF-2 ARE THE SAME MOLECULE

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VEGF-C      MHLLGFFSVACSLLAAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQL   60
VEGF-2      MHSLGFFSVACSLLAAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQL
                 *

VEGF-C      RSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILK  120
VEGF-2      RSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILK

VEGF-C      SIDNEWRKTQCMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSY  180
VEGF-2      SIDNEWRKTQCMPREVCIDVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSY

VEGF-C      LSKTLFEITVPLSQGPKPVTISFANHTSCRCMSKLDVYRQVHSIIRRSLPATLPQCQAAN  240
VEGF-2      LSKTLFEITVPLSQGPKPVTISFANHTSCRCMSKLDVYRQVHSIIRRSLPATLPQCQAAN

VEGF-C      KTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLR  300
VEGF-2      KTCPTNYMWNNHICRCLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLR

VEGF-C      PASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCAC  360
VEGF-2      PASCGPHKELDRNSCQCVCKNKLFPSQCGANREFDENTCQCVCKRTCPRNQPLNPGKCAC

VEGF-C      ECTESPQKCLLKGKKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCVPSYWKRPQMS  419
VEGF-2      ECTESPQKCLLKGKKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCVPSYWQRPQMS
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The consensus line:
* = Indicates substitutions that are neither conserved nor semi-conserved.
: = indicates conserved substitutions.
. = indicates semi-conserved substitutions.

AARONSON DECLARATION

APPENDIX I